PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 10 May 2001 (10.05.01)	
International application No. PCT/NZ00/00176	**Applicant's or agent's file reference** 26329 MRB
International filing date (day/month/year) 07 September 2000 (07.09.00)	**Priority date** (day/month/year) 07 September 1999 (07.09.99)
Applicant YAO, Jialong et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 March 2001 (05.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: P826329 TVG

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/NZ00/00176	7 September 2000(7/9/00)	7 September 1999 (7/9/99)

Title of invention: SEEDLESS FRUIT PRODUCTION

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

THE HORTICULTURE AND FOOD RESEARCH
INSTITUTE OF NEW ZEALAND LIMITED
Batchelar Research Centre
Highway 57
Palmerston North
New Zealand

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
New Zealand	New Zealand

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

YAO, Jialong
35 McFadzean Drive
Blockhouse Bay
Auckland
New Zealand

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
New Zealand	New Zealand

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MORRIS, Bret A
22 Pokapu Street
Green Bay
Auckland
New Zealand

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
New Zealand	New Zealand

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/NZ00/00176

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition t the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

A J PARK: CALHOUN, Douglas C; CHRISTIE, Andrew L; GRIFFITHS, Teresa V; JONES, David J; MOON, Kenneth R; SYDDALL, Thomas H; THOMSON, Keith C; and WEST-WALKER, Gregory J;
all of 6th Floor, Huddart Parker Building, Post Office Square, P O Box 949, Wellington 6015, New Zealand

Telephone No.: +64 4 473-8278

Facsimile No.: +64 4 472-3358

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[X] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [X] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

B x No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 3

International application No.
PCT/NZ00/00176

Box N . VI CHECK LIST

The demand is accompanied by the following elements, in the language referred t in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [X] other *(specify)*: Letter

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

TERESA VIDETTE GRIFFITHS
Agent for the Applicants

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand f r international preliminary examinati n

International application No. PCT/NZ00/00176	For International Preliminary Examining Authority use only
Applicant's or agent's file reference P826329 TVG	Date stamp of the IPEA

Applicant
THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED

Calculation of prescribed fees

1. Preliminary examination fee	AUD450.00	P
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	AUD238.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	AUD688.00	TOTAL

Mode of Payment

☐	authorization to charge deposit account with the IPEA (see below)	☐	cash
☐	cheque	☐	revenue stamps
☐	postal money order	☐	coupons
☐	bank draft	☒	other *(specify):* MasterCard

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ _____ ☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number	Date *(day/month/year)*	Signature

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 26329 MRB

Box No. I TITLE OF INVENTION

SEEDLESS FRUIT PRODUCTION 7/9/00

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED
Batchelar Research Centre
Highway 57
Palmerston North
New Zealand

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: NZ

State *(that is, country)* of residence: NZ

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

YAO, Jialong
35 McFadzean Drive
Blockhouse Bay
Auckland
New Zealand

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: NZ

State *(that is, country)* of residence: NZ

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BENNETT, Michael Roy; WEST-WALKER, Gregory James; RUTLEDGE, Sue Moira; ADAMS, Matthew Dickson of WEST-WALKER BENNETT
Mobil on the Park
157 Lambton Quay
Wellington, New Zealand

Telephone No. +64 4 499 9058

Facsimile No. +64 4 499 9306

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. . 2 . . . .

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MORRIS, Bret A
22 Pokapu Street
Green Bay
Auckland
New Zealand

This person is:

☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: NZ

State *(that is, country)* of residence: NZ

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] **AE** United Arab Emirates
- [X] **AG** Antigua and Barbuda
- [X] **AL** Albania .....
- [X] **AM** Armenia .....
- [X] **AT** Austria .....
- [X] **AU** Australia .....
- [X] **AZ** Azerbaijan
- [X] **BA** Bosnia and Herzegovina .....
- [X] **BB** Barbados
- [X] **BG** Bulgaria .....
- [X] **BR** Brazil .....
- [X] **BY** Belarus .....
- [X] **BZ** Belize
- [X] **CA** Canada
- [X] **CH and LI** Switzerland and Liechtenstein
- [X] **CN** China .....
- [X] **CR** Costa Rica .....
- [X] **CU** Cuba .....
- [X] **CZ** Czech Republic .....
- [X] **DE** Germany .....
- [X] **DK** Denmark .....
- [X] **DM** Dominica
- [X] **DZ** Algeria .....
- [X] **EE** Estonia .....
- [X] **ES** Spain .....
- [X] **FI** Finland .....
- [X] **GB** United Kingdom
- [X] **GD** Grenada
- [X] **GE** Georgia .....
- [X] **GH** Ghana .....
- [X] **GM** Gambia
- [X] **HR** Croatia .....
- [X] **HU** Hungary .....
- [X] **ID** Indonesia
- [X] **IL** Israel .....
- [X] **IN** India .....
- [X] **IS** Iceland
- [X] **JP** Japan .....
- [X] **KE** Kenya .....
- [X] **KG** Kyrgyzstan .....
- [X] **KP** Democratic People's Republic of Korea .....
- [X] **KR** Republic of Korea .....
- [X] **KZ** Kazakhstan .....
- [X] **LC** Saint Lucia
- [X] **LK** Sri Lanka
- [X] **LR** Liberia
- [X] **LS** Lesotho .....
- [X] **LT** Lithuania
- [X] **LU** Luxembourg
- [X] **LV** Latvia
- [X] **MA** Morocco .....
- [X] **MD** Republic of Moldova .....
- [X] **MG** Madagascar .....
- [X] **MK** The former Yugoslav Republic of Macedonia .....
- [X] **MN** Mongolia
- [X] **MW** Malawi .....
- [X] **MX** Mexico .....
- [X] **MZ** Mozambique
- [X] **NO** Norway
- [X] **NZ** New Zealand .....
- [X] **PL** Poland .....
- [X] **PT** Portugal .....
- [X] **RO** Romania
- [X] **RU** Russian Federation .....
- [X] **SD** Sudan
- [X] **SE** Sweden
- [X] **SG** Singapore
- [X] **SI** Slovenia .....
- [X] **SK** Slovakia .....
- [X] **SL** Sierra Leone .....
- [X] **TJ** Tajikistan .....
- [X] **TM** Turkmenistan .....
- [X] **TR** Turkey .....
- [X] **TT** Trinidad and Tobago .....
- [X] **TZ** United Republic of Tanzania
- [X] **UA** Ukraine .....
- [X] **UG** Uganda .....
- [X] **US** United States of America .....
- [X] **UZ** Uzbekistan .....
- [X] **VN** Viet Nam .....
- [X] **YU** Yugoslavia .....
- [X] **ZA** South Africa .....
- [X] **ZW** Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

- [ ] .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI. PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (07/09/1999) 7 September 1999	NZ 337688	NZ		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)* : ISA/AU

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	4
description (excluding sequence listing part)	:	24
claims	:	4
abstract	:	1
drawings	:	4
sequence listing part of description	:	7
Total number of sheets	:	44

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

MICHAEL ROY BENNETT
Agent for the Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: 26329 MRB

Applicant: THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . $180.00 [T]

2. SEARCH FEE . . . . . $990.00 [S]

International search to be carried out by Australian Patent Office

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 44 sheets.

first 30 sheets . . . . . $822.00 [b1]

14 (remaining sheets) x $19 (additional amount) = $266.00 [b2]

Add amounts entered at b1 and b2 and enter total at B . . . . $1088.00 [B]

Designation Fees

The international application contains 108 designations.

108 (number of designation fees payable *(maximum 8)*) x $178 (amount of designation fee) = $1424.00 [D]

Add amounts entered at B and D and enter total at I . . . . . $2512.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . – [P]

5. TOTAL FEES PAYABLE . . . . . $3682.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

[X] The designation fees are not paid at this time.

MODE OF PAYMENT

- [ ] authorization to charge deposit account (see below)
- [ ] bank draft
- [ ] coupons
- [ ] cheque
- [ ] cash
- [ ] other *(specify):*
- [ ] postal money order
- [ ] revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ _____ [ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[ ] *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

[ ] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No. | Date *(day/month/year)* | Signature

See Notes to the fee calculation sheet

kla

14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 20 APR 2001
WIPO PCT

Applicant's or agent's file reference 26329MRB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ00/00176**	International Filing Date *(day/month/year)* 7 September 2000	Priority Date *(day/month/year)* 7 September 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] A01H 5/08, C12N 15/29

Applicant

THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
5 March 2001	4 April 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **PHILIPPA WYRDEMAN** Telephone No. (02) 6283 2554

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
[redacted]T/NZ00/00176

I. Basis of the rep rt

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reas ned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanati ns supp rting such statement

1. Statement

Novelty (N)	Claims **1-34**	**YES**
	Claims **None**	**NO**
Inventive step (IS)	Claims **1-34**	**YES**
	Claims **None**	**NO**
Industrial applicability (IA)	Claims **1-34**	**YES**
	Claims **None**	**NO**

2. Citations and explanations (Rule 70.7)

Novelty (N)

All the documents cited in the ISR were category A only. Therefore the claimed invention is not disclosed in any of these patent documents and hence all the claims are novel.

Inventive Step (IS)

The claimed invention is not obvious in the light of any of the cited documents nor disclosed in any obvious combination, nor would the claimed invention be obvious to a person skilled in the art in the light of common general knowledge by itself or in combination with any of these documents.

Industrial Applicability (IA)

The claimed material is considered to be Industrially Applicable.

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(54) Title: SEEDLESS FRUIT PRODUCTION

(57) Abstract: The invention provides fruiting plants that produce seedless or sterile fruit. The production of seedless or sterile fruit is the result of genetic modification which prevents or disrupts functional expression of the *MdPI* peptide of SEQ ID NO: 2 or a variant thereof, or of the *MdAP3* peptide of SEQ ID NO: 4 or a variant thereof, or both.

SEEDLESS FRUIT PRODUCTION

FIELD OF THE INVENTION

The invention provides plants that produce seedless or sterile fruit.

BACKGROUND TO THE INVENTION

The production of seedless or parthenocarpic fruit is a desirable trait for commercially grown cultivars. Seedless fruit are more convenient than seeded fruit to consumers. Furthermore parthenocarpic fruit trees can be cropped without pollination, which reduces dependence on bees, pollinator varieties and warm weather at flowering. The absence of pollen is also advantageous so as to alleviate environmental concerns regarding the transfer of transgenes to non-transgenics by cross-pollination.

Seedless fruit cultivars can also avoid or reduce biennial bearing tendencies that have been attributed to the inhibition of flower bud formation by developing seeds in apple (Chan and Cain, 1967). Seedless apple fruit is also much less susceptible to codling moth, a major pest on apple trees, compared to seeded fruit (Goonewardene *et al.*, 1984).

The applicants have now identified and isolated a reproductive gene which encodes a peptide involved in the reproductive (seed-producing) cycle of fruiting plants, particularly apple trees. It is broadly towards this gene, to its homologs in other fruiting plants and to the modulation of its expression/function within fruiting plants that the present invention is directed.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a fruiting plant which has been genetically modified such that it does not functionally express:

(i) a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and/or

(ii) a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

which plant produces seedless or sterile fruit.

In a further aspect, the invention provides a fruiting plant which contains a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof and in which the functional expression of said peptide within said plant has been disrupted such that the plant produces seedless or sterile fruit.

In still a further aspect, the invention provides a fruiting plant which contains:

(a) a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(b) a polynucleotide encoding a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

and in which the functional expression of said peptide encoded by polynucleotide (a) within said plant has been disrupted such that the plant produces seedless or sterile fruit.

In one form, functional expression of said peptide encoded by polynucleotide (a) is disrupted directly.

In another form, functional expression of said peptide encoded by polynculeotide (a) is disrupted indirectly, such as through disrupting functional expression of the peptide encoded by said polynucleotide (b).

As used herein, "fruiting plant" means a plant in which the fruit is formed from the ovary and the fused bases of sepals, petals and stamen, whereas "functional

expression" of said peptide refers to the amount of the peptide which is expressed and functional within the plant. For example, a plant which does not functionally express a peptide can mean either that there is no expression of that peptide at all, or that the peptide is expressed but no longer performs its previous function.

Conveniently, the plant is one which produces a pome fruit.

Disruption of functional expression may be by mutation (such as frameshift, deletion, insertion or knockout mutations) of the gene itself or of its regulatory elements, down-regulation (such as antisense, co-suppression) or any other method known to those skilled in the art by which aberrant or reduced expression of the gene may be achieved (e.g. Montgomery and Fire, 1998).

Disruption may therefore be specifically caused by down-regulation of expression of *MdPI* by down-regulation of expression of inter-related *MdAP3*, or both.

In a further embodiment, the invention provides a polynucleotide which encodes a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a variant thereof, or which encodes a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a variant thereof.

Most preferably, said polynucleotide includes part or all of the nucleotide sequence of SEQ ID NO: 1, or part or all of the nucleotide sequence of SEQ ID NO: 3.

Preferably, the polynucleotide is DNA.

The invention further provides a DNA construct which includes a polynucleotide as defined above.

More particularly, the invention provides a DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) an open reading frame polynucleotide coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(c) a termination sequence.

In one embodiment, the open reading frame is in a sense orientation.

In an alternative embodiment, the open reading frame is in an anti-sense orientation.

In still a further embodiment, the invention provides a DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a non-coding region of a gene coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(c) a termination sequence.

Once again, the non-coding region can be in a sense or anti-sense orientation.

In yet a further embodiment, the invention provides a DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a polynucleotide comprising a nucleotide sequence complementary to at least part of a sequence coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(c) a termination sequence.

Preferably, in each embodiment, the construct further includes a marker for identification of transformed cells.

Similar constructs can also be provided including a polynucleotide which encodes part or all of the *MdAP3* peptide having the sequence of SEQ ID NO: 4.

In still a further aspect, the invention provides a transgenic fruiting plant cell which includes a DNA construct as defined above, as well as a transgenic fruiting plant comprising such cells.

Finally, the invention includes seedless or sterile fruit produced by a plant as defined above.

DESCRIPTION OF THE DRAWINGS

While the invention is broadly defined as above, those persons skilled in the art will appreciate that it is not limited thereto and that it also includes embodiments of which the following description provides examples or which are the subject of specific claims. In addition, the present invention will be better understood from reference to the accompanying drawings in which:

Figure 1 shows the phenotype of wild type and Rae Ime apple flowers and fruit.

(a) normal apple flowers showing sepals, petals, stamens and styles.

(b) a normal 5-week-old apple fruit showing five carpels with 0 to 2 seeds per carpel.

(c) Rae Ime flowers with no petals or stamens but with increased numbers of styles.

(d) cross sections at the lower part (left) and upper part of a 5-week-old Rae Ime fruit, showing two whorls of carpels without seed.

(e) top of Rae Ime fruit showing two whorls of calyxes.

(f) top of normal apple fruit showing a whorl of calyxes.

(g) mature fruit of Rae Ime with size of 5 cm wide and no seed.

Figure 2 shows the sequence of *MdPI*. The cDNA sequences and deduced amino acid sequences of *MdPI* isolated from Granny Smith apple are shown. Gene specific PCR primers are underlined. Primer directions are indicated with horizontal arrows. Intron positions are indicated with vertical arrows.

Figure 3 shows a Northern blot analysis of apple RNA sample using *MdPI* cDNA as a probe. RNA sample were prepare from ovaries (1), sepals (2), young leaves (3), skin (4), cortex (5) and core (6) tissue of 4-week-old fruit of Granny Smith, 1-week-old fruit (7), flower peduncles (8), stamens (9), petals (10) of Granny Smith (12), flower buds of Rae Ime (11), and flower buds of Granny Smith (12).

Figure 4 shows a Southern analysis of apple genomic DNA using *MdPI* cDNA as a probe. DNA of Rae Ime (Ri) and Granny Smith (Gs) were digested with EcoRI (E) and HindIII (H).

Figure 5 shows the identification of a transposon insertion in *MdPI* of Rae Ime, Spencer Seedless and Wellington Bloomless.

(a) Genomic DNA fragments were amplified using primers P3 and P7 from Rae Ime (Ri) and Granny Smith (Gs).

(b) Southern blot made from the gel shown in (a) was probed with the cDNA of *MdPI*.

(c) The genomic DNA of *MdPI* from Granny Smith, Rae Ime, Spencer Seedless and Wellington Bloomless was sequenced. The sequence of *MdPI* of Granny Smith was numbered from the ATG start codon. The black boxes are the coding regions and the white box is the 3' non-coding region. A transposon insertion was found in the intron 4 of *MdPI* of Rae Ime and in the intron 6 of Spencer Seedless (Ss) and Wellington Bloomless (Wb) as shown by the arrows.

Figure 6 shows the cDNA and deduced amino acid sequences of *MdAP3*.

DESCRIPTION OF THE INVENTION

As broadly outlined above, the applicants have identified a peptide which is involved in fruiting plant reproduction, together with the gene coding therefor. The

specific peptide and gene are from a plant which produces pome fruit, *Malus x domestica*.

The amino acid sequence of one peptide, *MdPI*, and its encoding nucleotide sequence are given in Figure 2. It will however be appreciated that the invention is not restricted only to the peptide/polynucleotide having the specific amino acid/nucleotide sequence given in Figure 2. Instead, the invention also extends to functionally equivalent variants of the peptide/polynucleotide of Figure 2.

The term "polynucleotide(s)" as used herein means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

The phrase "functionally equivalent variants" recognises that it is possible to vary the amino acid/nucleotide sequence of a peptide while retaining substantially equivalent functionality. For example, a peptide can be considered a functional equivalent of another peptide for a specific function if the equivalent peptide is immunologically cross-reactive with and has at least substantially the same function as the original peptide. The equivalent can be, for example, a fragment of the peptide, a fusion of the peptide with another peptide or carrier, or a fusion of a fragment which additional amino acids.

It will of course be understood that a variety of substitutions of amino acids is possible while preserving the structure responsible for activity of the peptide. Conservative substitutions are described in the patent literature, as for example, in United States Patent No 5,264,558 or 5,487,983. It is thus expected, for example, that interchange among n n-polar aliphatic neutral amino acids, glycine, alanine,

proline, valine and isoleucine, would be possible. Likewise, substitutions among the polar aliphatic neutral amino acids, serine, threonine, methionine, asparagine and glutamine could be made. Substitutions among the charged acidic amino acids, aspartic acid and glutamic acid, could probably be made, as could substitutions among the charges basic amino acids, lysine and arginine. Substitutions among the aromatic amino acids, including phenylalanine, histidine, tryptophan and tyrosine are also possible. Such substitutions and interchanges are well known to those skilled in the art.

Equally, nucleotide sequences encoding a particular product can vary significantly simply due to the degeneracy of the nucleic acid code.

Variants can have a greater or lesser degree of homology as between the variant amino acid/nucleotide sequence and the original.

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another sequence, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (ftp://ncbi.nlm.nih.gov) under /blast/executables/. The BLASTN algorithm version 2.0.4 [Feb-24-1998], set to the default parameters described in the documentation of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL http://www.ncbi.nlm.nih.gov/BLAST/newblast.html and in the publication of Altschul, Stephen F., *et al.* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-34023. The computer algorithm FASTA is available on the Internet at the ftp site ftp://ftp.virginia.edu/pub/fasta/. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is also preferred for use in the determination of variants according to the present invention. The use of the FASTA algorithm is described in W. R. Pearson and D. J. Lipman, "Improved Tools for Biological Sequence Analysis", *Proc.*

Natl. Acad. Sci. USA 85:2444-2448 (1988) and W. R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA, "*Methods in Enzymology* 183:63-98 (1990).

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to E values (as discussed below) and percentage identity: Unix running command: blastall -p blastn -d embldb -e 10 -G 1 -E 1 -r 2 -v 50 -b 50 -I queryseq -o results; and parameter default values:

-p Program Name [String]

-d Database [String]

-e Expectation value (E) [Real]

-G Cost to open a gap (zero invokes default behaviour) [Integer]

-E Cost to extend a cap (zero invokes default behaviour) [Integer]

-r Reward for a nucleotide match (blastn only) [Integer]

-v Number of one-line descriptions (V) [Integer]

-b Number of alignments to show (B) [Integer]

-i Query File [File In]

-o BLAST report Output File [File Out] Optional

For BLASTP the following running parameters are preferred: blastall -p blastp -d swissprotdb -e 10 -G 1 -E 1 -v 50 -b 50 -I queryseq -o results

-p Program Name [String]

-d Database [String]

-e Expectation value (E) [Real]

-G Cost to open a gap (zero invokes default behaviour) [Integer]

-E Cost to extend a cap (zero invokes default behaviour) [Integer]

-v Number of one-line descriptions (v) [Integer]

-b Number of alignments to show (b) [Integer]

-i Query File [File In]

-o BLAST report Output File [File Out] Optional

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN and FASTA algorithms also produce "Expect" or E values for alignments. The E value indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a 90% probability of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides, with reference to each of the polynucleotides of the present invention, preferably comprise sequences having the same number or fewer nucleic acids than each of the polynucleotides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide of the present invention. That is, a variant polynucleotide is any sequence that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the parameters discussed above.

Variant polynucleotide sequences will generally hybridize to the recited polynucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

It is of course expressly contemplated that homologs to *MdPI* exist in other fruiting plants. Such homologs are also "functionally equivalent variants" of *MdPI* as the phrase is used herein.

DNA sequences from fruiting plants other than *Malus x domestica* which are homologs of *MdPI* may be isolated by high throughput sequencing of cDNA libraries prepared from such plants. Alternatively, oligonucleotide probes based on the sequences for *MdPI* provided in Figure 2 can be synthesized and used to identify positive clones in either cDNA or genomic DNA libraries from other plants by means of hybridization or PCR techniques. Probes should be at least about 10, preferably at least about 15 and most preferably at least about 20 nucleotides in length. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art. Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

The polynucleotides of the present invention may be generated by synthetic means using techniques well known in the art. Equipment for automated synthesis of oligonucleotides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems Division (Foster City, CA) and may be operated according to the manufacturer's instructions.

The primary importance of identification of the peptide/polynucleotides of the invention is that they enable the reproductive (seed-producing) capacity of fruiting plants to be modulated. This modulation will generally involve a reduction in the functional expression (silencing) of the reproductive peptide.

Any conventional technique for effecting this can be employed. Intervention can occur post-transcriptionally or pre-transcriptionally. Further, intervention can be focused upon the gene itself or on regulatory elements associated with the gene and which have an effect on expression of the encoded peptide. "Regulatory elements" is used here in the widest possible sense and includes other genes which interact with the gene of interest. For example, intervention which targets expression of *MdAP3* peptide is contemplated. *MdAP3* is functionally related to *MdPI* such that down-regulation of *MdAP3* expression will in turn down-regulate *MdPI* (see Jack *et al* (1992) and Goto & Meyerowitz (1994)).

The cDNA and deduced amino acid sequences for *MdAP3* are shown in Figure 6.

Pre-transcription intervention can involve mutation of the gene itself or of its regulatory elements. Such mutations can be point mutations, frameshift mutations, insertion mutations or deletion mutations. These latter mutations include so call "knock-out" mutations in which the gene is entirely ablated.

Examples of post-transcription interventions include co-suppression or anti-sense strategies, a dominant negative approach, or techniques which involve ribozymes to digest, or otherwise be lethal to, RNA post-transcription of the target gene.

Co-suppression can be effected in a manner similar to that discussed, for example, by Napoli *et al* (Plant Cell 2:279-290, 1990) and de Carvalho Niebel *et al* (Plant Cell 7:347-258, 1995). In some cases, it can involve overexpression of the gene of interest through use of a constitutive promoter. It can also involve transformation of a plant with a non-coding region of the gene, such as an intron from the gene or 5'-non-coding leader sequences.

Anti-sense strategies involve expression or transcription of DNA with the expression/transcription product being capable of interfering with translation of mRNA transcribed from the target gene. This will normally be through the expression/transcription product hybridising to and forming a duplex with the target mRNA.

The expression/transcription product can be a relatively small molecule and still be capable of disrupting mRNA translation. However, the same result is achieved by expressing the target gene in an anti-sense orientation such that the RNA produced by transcription of the anti-sense oriented gene is complementary to all or part of the endogenous target mRNA.

Anti-sense strategies are described generally by Robinson-Benion *et al.,* (1995), Anti-sense techniques, *Methods in Enzymol.* 254(23):363-375 and Kawasaki *et al.*, (1996), *Artific. Organs* 20 (8): 836-848.

Dominant negative approaches involve the expression of a modified DNA binding/activating protein which includes a DNA binding domain but not a activator domain. The result is that the protein binds to DNA as intended but fails

to activate, while at the same time blocking the binding of the DNA binding/activating peptides which normally bind to the same site.

The ribozyme approach to regulation of peptide expression involves inserting appropriate sequences or subsequences (eg. DNA or RNA) in ribozyme constructs (McIntyre CL, Manners JM, *Transgenic Res.* 5(4):257-262, 1996). Ribozymes are synthetic RNA molecules that comprise a hybridizing region complementary to two regions, each of which comprises at least 5 contiguous nucleotides of a mRNA molecule encoded by one of the inventive polynucleotides. Ribozymes possess highly specific endonuclease activity, which autocatalytically cleaves the mRNA.

To give effect to the above strategies, the invention also provides DNA constructs. The constructs include the intended DNA (such as the gene of the invention in anti-sense orientation or a polynucleotide encoding the appropriate DNA binding domain or ribozyme), a promoter sequence and a termination sequence, operably linked to the DNA sequence to be transcribed, which control expression of the gene. The promoter sequence is generally positioned at the 5' end of the DNA sequence to be transcribed, and is employed to initiate transcription of the DNA sequence. Promoter sequences are generally found in the 5' non-coding region of a gene but they may exist in introns (Luehrsen, K.R., *Mol. Gen. Genet.* 225:81-93, 1991) or in the coding region. When the construct includes an open reading frame in a sense orientation (for co-suppression through over-expression) the promoter sequence also initiates translation of the open reading frame. For DNA constructs comprising either an open reading frame in an anti-sense orientation or a non-coding region, the promoter sequence generally consists only of a transcription initiation site having a RNA polymerase binding site.

A variety of promoter sequences which may be usefully employed in the DNA constructs of the present invention are well known in the art. The promoter sequence, and also the termination sequence, may be endogenous to the target *Malus* plant host or may be exogenous, provided the promoter is functional in the target host. For example, the promoter and termination sequences may be from other plant species, plant viruses, bacterial plasmids and the like. Preferably, promoter and termination sequences are those endogenously associated with the reproductive genes.

Factors influencing the choice of promoter include the desired tissue specificity of the construct, and the timing of transcription and translation. For example, constitutive promoters, such as the 35S Cauliflower Mosaic Virus (CaMV 35S) promoter, will affect the activity in all parts of the plant. Use of a tissue specific promoter will result in production of the desired sense or antisense RNA only in the tissue of interest. With DNA constructs employing inducible promoter sequences, the rate of RNA polymerase binding and initiation can be modulated by external stimuli, such as light, heat, anaerobic stress, alteration in nutrient conditions and the like. Temporally regulated promoters can be employed to effect modulation of the rate of RNA polymerase binding and initiation at a specific time during development of a transformed cell. Preferably, the original promoters from the gene in question, or promoters from a specific tissue-targeted gene in the organism to be transformed are used. Other examples of promoters which may be usefully employed in the present invention include, mannopine synthase (mas), octopine synthase (ocs) and those reviewed by Chua *et al.* (Science, 244:174-181, 1989).

The termination sequence, which is located 3' to the DNA sequence to be transcribed, may come from the same gene as the promoter sequence or may be from a different gene. Many termination sequences known in the art may be usefully employed in the present invention, such as the 3' end of the *Agrobacterium tumefaciens* nopaline synthase gene. However, preferred termination sequences are those from the original gene or from the target *Malus* species to be transformed.

The DNA constructs of the present invention may also contain a selection marker that is effective in plant cells, to allow for the detection of transformed cells containing the construct. Such markers, which are well known in the art, typically confer resistance to one or more toxins. One example of such a marker is the NPTII gene whose expression results in resistance to kanamycin or hygromycin, antibiotics which is usually toxic to plant cells at a moderate concentration (Rogers *et al.*, in *Methods for Plant Molecular Biology*, A Weissbach and H Weissbach eds, Academic Press Inc., San Diego, CA (1988)). Alternatively, the presence of the

desired construct in transformed cells can be determined by means of other techniques well known in the art, such as Southern and Western blots.

Techniques for operatively linking the components of the inventive DNA constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Maniatis *et al.*, (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratories, Cold Spring Harbour, NY, 1989). The DNA construct may be linked to a vector having at least one replication system, for example, *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

The DNA constructs of the present invention may be used to transform a variety of fruiting plants. In a preferred embodiment, the DNA constructs are employed to transform apple and its related species such as pear.

As discussed above, transformation of a fruiting plant with a DNA construct including an open reading frame coding for a peptide encoded by a DNA sequence of the invention wherein the open reading frame is orientated in a sense direction can, in some cases, lead to a decrease in expression of the peptide by co-suppression. Transformation of the plant with a DNA construct comprising an open reading frame in an anti-sense orientation or a non-coding (untranslated) region of a gene will lead to a decrease in the expression of the peptide in the transformed plant.

Techniques for stably incorporating DNA constructs into the genome of target fruiting plants are well known in the art and include *Agrobacterium tumefaciens* mediated introduction, electroporation, protoplast fusion, injection into reproductive organs, injection into immature embryos, high velocity projectile introduction and the like. The choice of technique will depend upon the target plant to be transformed.

Once the cells are transformed, cells having the DNA construct incorporated into their genome may be selected by means of a marker, such as the kanamycin resistance marker discussed above. Transgenic cells may then be cultured in an

appropriate medium to regenerate whole plants, using techniques well known in the art. In the case of protoplasts, the cell wall is allowed to reform under appropriate osmotic conditions. In the case of seeds or embryos, an appropriate germination or callus initation medium is employed. For explants, an appropriate regeneration medium is used.

For a review of regeneration of trees, see Dunstan *et al.*, Somatic embryogenesis in woody plants. In: Thorpe, T.A. ed. 1995: *in vitro* embryogenesis of plants. Vol 20 in Current Plant Science and Biotechnology in Agriculture, Chapter 12, pp. 471-540.

The resulting transformed fruiting plants may be reproduced sexually or asexually, using methods well known in the art, to give successive generations of transgenic plants.

The nucleotide sequence information provided herein will also be useful in programs for identifying nucleic acid variants from fruiting plants and for pre-selecting plants with mutations in *MdPI*, *MdAP3* or their equivalents which renders those plants useful in an accelerated breeding program to produce seedless fruit. More particularly, the nucleotide sequence information provided herein may be used to design probes and primers for probing or amplification of *MdPI*, *MdAP3* or variants thereof. An oligonucleotide for use in probing or PCR may be about 30 or fewer nucleotides in length. Generally, specific primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers or 16-24 nucleotides in length are preferred. Those skilled in the art are well versed in the design of primers for use in processes such as PCR.

If required, probing can be done with entire restriction fragments of the gene disclosed herein. Naturally, sequences based upon Figure 2, or Figure 6 or the complements thereof can be used.

Such probes and primers also form aspects of the present invention.

Probing may employ the standard Southern blotting technique. For instance, DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel,

before denaturation and transfer to a nitrocellulose filter. Labelled probes may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells. Probing may optionally be done by means of so-called "nucleic acid chips" (see Marshall and Hodgson (1998)).

The invention will now be illustrated with reference to the following non-limiting experiments.

EXPERIMENTAL

Methods and Materials

Cloning MdPI using PCR approaches

Total RNA was isolated from 'Granny Smith' apple flowers using the method described by Chang *et al* (1993). Poly(A) mRNA was purified from the total RNA using the mRNA Purification Kit (Pharmacia, Sweden). cDNA was synthesized from the mRNA using the ZAP cDNA Synthesis Kit (Stratagene, CA, USA). DNA fragments were amplified from templates of cDNA using two degenerative PCR primers P1 CGGAATTCATGGGNMGNGGNAARRT-3' and P2 CGCTCGAGGATCCGGYTGNATNGGYTGNAC-3' (N=ATGC, M=AC, R=AG, Y=CT). The primers were designed according the conserved amino acid sequences MGRGKI in the MADS-box domain and VQPM/IQP in the C-terminal region (Fig. 2) in an alignment of PI, GLOBSA, FBP3, SLM2 and pMADS2. The underlined Eco RI and Bam HI sites were used for cloning the PCR products. The PCR amplification conditions were as follows: initial denaturation at 94°C for 4 min; then 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 3 min, and with a final extension of 5 min at 72°C. Several bands were detected from the PCR on agarose gels and DNA in a band of the expected size (630 bp) was cloned into Bluescript SK (Stratagene, CA, USA) following Eco RI and Bam HI digestion. After the sequences of cloned fragments were determined, two nested PCR primers, P3 and P4 (Fig. 2) were designed using the sequences within the K-box and were used to amplify the 3' region of MdPI cDNA together with a 3' RACE primer GAGAGAGAACTAGTCTCGAG-3'. The PCR conditions were the same as above except for the anneal temperature

reduced to 50°C. The amplified fragments were cloned into pGEM-T EASY Vector (Promega).

Genomic fragments of MdPI were amplified using primers P5 and P6, P3 and P7 (Fig. 2). PCR conditions were: initial denaturation at 94°C for 2 min; then 10 cycles of 94°C for 15 sec, 58°C for 30 sec; and 20 cycles of 94°C for 15 sec, 58°C for 30 sec and 68°C for 5 min plus cycle elongation of 20 sec for each cycle; and with a final extension of 5 min at 86°C. The amplified fragments were cloned into pGEM-T EASY Vector. Expand High Fidelity PCR System (Boehringer Mannheim) was used for all PCR experiments.

DNA sequence determination

Nucleotide sequences of MdPI clones were determined using the automatic sequencer ABI PRISM model 377(CA, USA) with universal forward and reverse primers. To obtain complete sequences, gene specific primers were designed and ordered from BRL Life Technologies.

Northern and Southern analysis using MdPI on apple tissues

Total RNA was isolated as described by Chang *et al* (1993) from 'Granny Smith' and Rae Ime apple tissues. Northern blots were prepared as described by Dong *et al* (1997). The northern blot contained RNA isolated from expanding leaves, unopened flowers, and fruit at 2 days and 1, 4 and 8 weeks following hand-pollination. At 4 weeks after pollination, apple fruit is large enough to allow for easy separation into the three main tissue types namely; core, cortex and skin.

DNA was isolated from leaf tissue of Granny Smith and Rae Ime using the method of Rogers and Bendich (1988). Southern blots were prepared by digesting apple DNA (approximately 20 µg per lane) with EcoRI or HindIII, separating DNA fragments on 0.7% agarose gel and transferring them to Hybond-N+ membrane.

Northern and Southern blots were probed with 32P-dCTP labelled PI cDNA clone lacking the MADS-box sequence. to significantly reduce cross hybridization32P-dCTP labelled MADS-box DNA fragments. The blots were hybridized in 0.5M NaPO4 buffer (pH 7.2) with 1 mM EDTA and 7% SDS at 65°C and washed using 0.4x SSC

and 0.2% SDS at 65°C. Hybridisation signals were detected using a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, California, USA).

Results/Discussion

Flowers of the majority of apple taxa bear 5 sepals, 5 petals, 9-20 stamens (Fig. 1a) and an inferior ovary. These flowers develop into a pome fruit that consists of fleshy cortex tissue derived from the fused bases of sepals, petals and stamens, and the core tissue derived from fertilised ovary containing 5 carpels and up to 10 seeds (Pratt, 1988) (Fig. 1b). In contrast, flowers of Rae Ime show no petal or stamens but increased numbers of styles (Fig. 1c). These flowers develop into seedless fruit without the need for pollination. These seedless fruit have two whorls of carpels, five carpels in the lower whorl and 9 to 10 in the upper whorl (Fig. 1d). The fruit also has duplicated whorls of calyxes (Fig. 1e) that are the remains of sepals, compared to one calyx whorl in a normal apple (Fig. 1f). The mature seedless fruit are close to normal apple fruit size, but the fruit cores are relatively smaller (Fig. 1g).

Several apple varieties, such as Spencer Seedless and Wellington Bloomless (Tobutt, 1994), have been described with a very similar flower and fruit structure to that of Rae Ime. Anatomy studies of the vascular connections show that the upper whorl of carpels has been transformed from the stamens and the second whorl of sepals from petals (Brase, 1937). In the *Arabidopsis pi* and *ap3* mutants, flowers have no petals or stamens but have double the number of sepals and carpels (Goto and Meyerowitz, 1994; Jack *et al.*, 1992).

A difference between Rae Ime apple and *pi Arabidopsis* is that the former produces parthenocarpic fruit but the latter does not. Up to 6 apple varieties have been recorded to produce apetalous flowers and parthenocarpic fruit in different countries. Many of these records can be traced back to several centuries ago (Brase, 1937; Tobutt, 1994). This indicates some of the apple mutants may have occurred independently.

Genetic analysis has been performed using two apetalous/parthenocarpic varieties, Spencer Seedless and Wellington Bloomless. Crossing pollen from the

cultivar Wijcik with normal flowers to Wellington Bloomless generates hybrids that all produce normal flowers. Crossing the pollen from these hybrids to Spencer Seedless generates plants of which half produce normal flowers and half produce apetalous flowers and parthenocarpic fruit (Tobutt, 1994). This result indicates that a single recessive gene controls apetalous flower development and subsequently parthenocarpic fruit formation. This result also indicates that mutations in Spencer Seedless and Wellington Bloomless are different alleles at the same locus. Independently isolated mutant alleles at the same locus are good evidences for a single gene being involved in the development of apetalous flower and parthenocarpic fruit in these apple mutants.

DNA fragments of 630bp have been amplified from apple flower cDNA using degenerative PCR primers against conserved sequences in the MADS-box and in the C-terminal region of *PI* and its homologues. After these DNA fragments were cloned, 6 random clones were sequenced and found to contain the same sequences. The cloned cDNA sequences started from the first presumed ATG start coden, contained MADS-box, K-box and most of the C-terminal region and had high homology to *PI*. The C-terminal and the 3' un-translated regions were further amplified using two nested PCR primers within the K-box and a 3' RACE primer. Six clones containing the 3' fragments were sequenced and found to contain the same sequences overlapping with those in the 5' clone. Sequences from the 5' and 3' clone were assembled together and shown in Fig. 2. These sequences show highest homology to *PI* and its homologues (GLOBOSA, FBP3, SLM2 and pMADS2) in Blast searches carried out in GeneBank. The putative apple *PI* homologue was named as *MdPI* having a deduced amino acid sequence identity of 64% to that of *Arabidopsis* PI protein.

MdPI is found to be highly expressed in petals and stamens as determined through northern analysis. Expression in other apple tissues, including sepals and ovaries, is either not detected or found to be very low (Fig. 3). This expression pattern is essentially the same as that shown for *Arabidopsis PI* gene (Goto and Meyerowitz, 1993). Genomic sequences of *MdPI* were amplified using the PCR primers P5 within the MADS-box and P6 within the 3' non-translated region. Two clones containing the *MdPI* genomic DNA were sequenced and found to contain the same sequences having six easily identifiable introns. The relative positions of intron 2

to intron 6 are highly conserved compared to the positions of 5 introns in *PI* gene (Fig. 2). We conclude that *MdPI* is the *PI* homolog based on these results having highest sequence identity and conserved intron positions and mRNA expression patterns.

In an experiment to examine whether there is a mutation in *MdPI* of Rae Ime, the expression level of *MdPI* in flower buds was determined. Expression of *MdPI* in the apetalous Rae Ime flower buds is not detected, but is readily detected in normal flower buds of the Granny Smith variety (Fig. 3). In *Arabidopsis pi* mutants, *PI* expression is reduced or abolished in flower buds (Goto and Meyerowitz, 1994).

A second experiment compared RFLP patterns for Rae Ime with normal apple cultivars using the *MdPI* cDNA as a probe. Southern hybridisation shows different RFLP patterns between Rae Ime and Granny Smith with both *Eco*RI and *Hind*III digestion (Fig. 4) although Granny Smith RFLP pattern is conserved in another apple variety Royal Gala (data not shown). Both the expression and RFLP data indicate that the *MdPI* gene in Rae Ime has been mutated. As both enzyme digestions reveal RFLP differences, the mutation is likely to be a gross change in gene structure rather than a point mutation

Genomic DNA fragments were cloned from Granny Smith and Rae Ime using two primers P3 and P7 designed with *MdPI* cDNA sequence. The Rae Ime fragments were 11 kb while the Granny Smith fragments were 2 kb (Fig. 5a). These fragments show a hybridisation signal to the *MdPI* cDNA probe (Fig. 5b). Clones containing these fragments were partially sequenced from two ends. The Rae Ime fragments have the same sequence to the Granny Smith fragments at two ends, but with an insertion in the intron 4 of *MdPI* gene in Rae Ime (Fig. 5b). The insertion sequences were found to be an LTR retrotransposon. This result confirmed that there is a mutation in the *MdPI* gene in Rae Ime.

By way of confirmation that it is the mutation of the *MdPI* gene which is responsible for the parthenocarpic phenotype, the *MdPI* gene from two further parthenocarpic apple varieties, Spencer Seedless and Wellington Bloomless, was sequenced (data not shown). This revealed an approximately 9 kb insertion in each gene. Thus, in the three parthenocarpic apple varieties examined, there are

two different insertion sites in the *MdPI* gene both of which lead to the parthenocarpic phenotype. Spencer Seedless and Wellington Bloomless have the same insertion site, which is different from that in Rae Ime (Fig. 5c). These confirmatory results demonstrate that independent mutations in *MdPI* generate the same apetalous/parthenocarpic phenotype.

The difference in fruit development between Rae Ime apple and *pi Arabidopsis* may be explained in two different ways. Firstly, *MdPI* may have different function compared to *PI* in influencing ovary and fruit development. Sufficient functional differences have been shown for homologs of floral homeotic genes in different plant species (Causier *et al.*, 1999). Secondly, apple fruit develops from both ovary and the fused bases of sepals, petals and stamens (Pratt, 1988). Apple differs from tomato and *Arabidopsis*, two model systems often used in studies of fruit development, where the fruit or silique develops from ovary tissue only (Weigel and Mererowitz, 1994; Gillaspy *et al.*, 1993). The differences in fruit structure may cause different fruit development after a mutation in a floral homeotic gene.

INDUSTRIAL APPLICATION

In its primary aspect, the invention has application in modulating, and in particular reducing or eliminating seed-bearing capacity in fruiting plants. Such plants have utility in horticulture.

It will also be possible to employ the polynucleotides of the invention in breeding programmes to monitor the progress made towards breeding a stable seedless fruiting plant.

The availability of reproductively null or sterile trees has the additional advantage that it will be possible to introduce further exogenous genetic material into those trees without the risk that the material will be passed on to other trees.

Those persons skilled in the art will appreciate that the specific description provided is exemplary only, and that modifications and variations may be made without departing from the scope of the invention.

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CLAIMS:

1. A fruiting plant which has been genetically modified such that it does not functionally express:

   (i) a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and/or

   (ii) a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

   which plant produces seedless or sterile fruit.

2. A fruiting plant which contains a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof and in which the functional expression of said peptide within said plant has been disrupted such that the plant produces seedless or sterile fruit.

3. A fruiting plant according to claim 1 or claim 2 which produces a pome fruit.

4. A fruiting plant which contains:

   (a) a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

   (b) a polynucleotide encoding a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

   and in which the functional expression of said peptide encoded by polynucleotide (a) within said plant has been disrupted such that the plant produces seedless or sterile fruit.

5. A plant as claimed in claim 4 wherein functional expression of said peptide encoded by polynucleotide (a) is disrupted directly.

6. A plant as claimed in claim 4 wherein functional expression of said peptide encoded by polynculeotide (a) is disrupted indirectly.

7. A plant as claimed in claim 6 wherein said indirect disruption is effected through disrupting functional expression of the peptide encoded by said polynucleotide (b).

8. A plant as claimed in any one of claims 4 to 7 wherein said plant is one which produces pome fruit.

9. A plant as claimed in claim 8 wherein said polynucleotide (a) has the coding sequence of SEQ ID NO: 1.

10. A plant as claimed in claim 8 wherein said polynucleotide (a) has the nucleotide sequence of SEQ ID NO: 1.

11. A plant as claimed in claim 8, claim 9 or claim 10 in which said polynucleotide (b) has the coding sequence of SEQ ID NO: 3.

12. A plant as claimed in claim 8, claim 9 or claim 10 wherein said polynucleotide (b) has the nucleotide sequence of SEQ ID NO: 3.

13. A polynucleotide which encodes a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof.

14. A polynucleotide as claimed in claim 13 which comprises the coding sequence of SEQ ID NO: 1.

15. A polynucleotide as claimed in claim 13 which comprises the nucleotide sequence of SEQ ID NO: 1.

16. A polynucleotide which encodes a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof.

17. A polynucleotide as claimed in claim 16 which comprises the coding sequence of SEQ ID NO: 3.

18. A polynucleotide as claimed in claim 16 which comprises the nucleotide sequence of SEQ ID NO: 3.

19. A DNA construct which includes a polynucleotide as claimed in any one of claims 13 to 18.

20. A DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) an open reading frame polynucleotide as defined in any one of claims 13 to 18; and

(c) a termination sequence.

21. A DNA construct as claimed in claim 20 wherein the open reading frame polynucleotide is in a sense orientation.

22. A DNA construct as claimed in claim 20 in which the open reading frame polynucleotide is in an anti-sense orientation.

23. A DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a non-coding region of a gene coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(c) a termination sequence.

24. A DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a non-coding region of a gene coding for the peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof; and

(c) a termination sequence.

25. A DNA construct as claimed in claim 23 or claim 24 in which the non-coding region is in a sense orientation.

26. A DNA construct as claimed in claim 23 or claim 24 in which the non-coding region is in an anti-sense orientation.

27. A DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a polynucleotide comprising a nucleotide sequence complementary to at least part of a sequence coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and

(c) a termination sequence.

28. A DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

(b) a polynucleotide comprising a nucleotide sequence complementary to at least part of a sequence coding for the peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof; and

(c) a termination sequence.

29. A transgenic cell of a fruiting plant which includes a DNA construct as claimed in any one of claims 19 to 28.

30. A transgenic cell as claimed in claim 29 in which said fruiting plant is one which produces a pome fruit.

31. A fruiting plant containing a transgenic cell as claimed in claim 29.

32. A fruiting plant containing a transgenic cell as claimed in claim 30.

33. A seedless or sterile fruit which is produced by a fruiting plant as claimed in any one of claims 1, 2, 4-7 and 31.

34. A seedless or sterile pome fruit which is produced by a fruiting plant as claimed in any one of claims 3, 8 to 12 and 32.

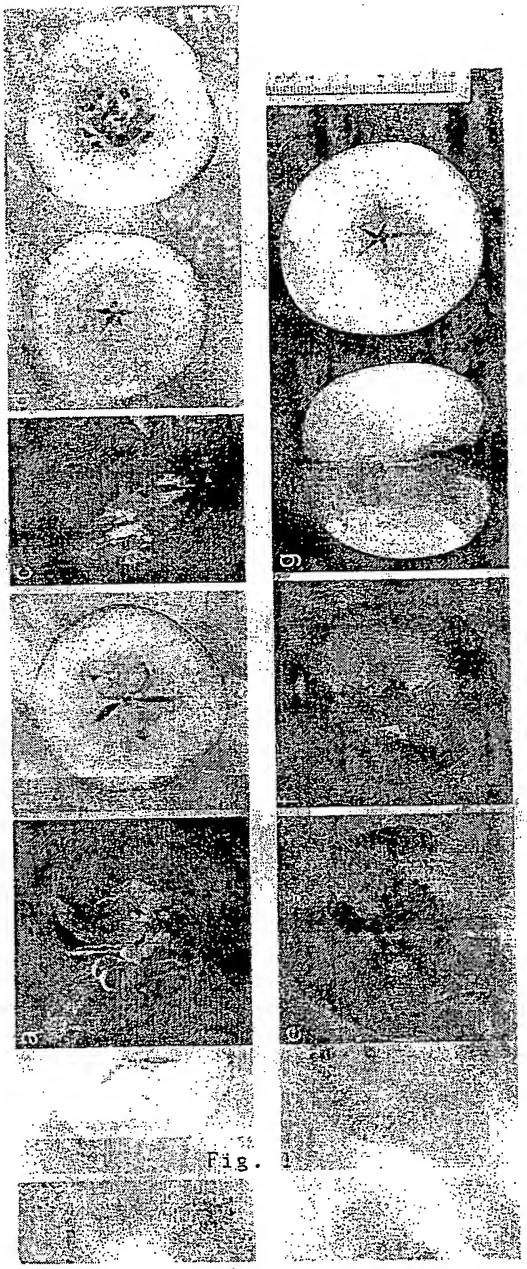
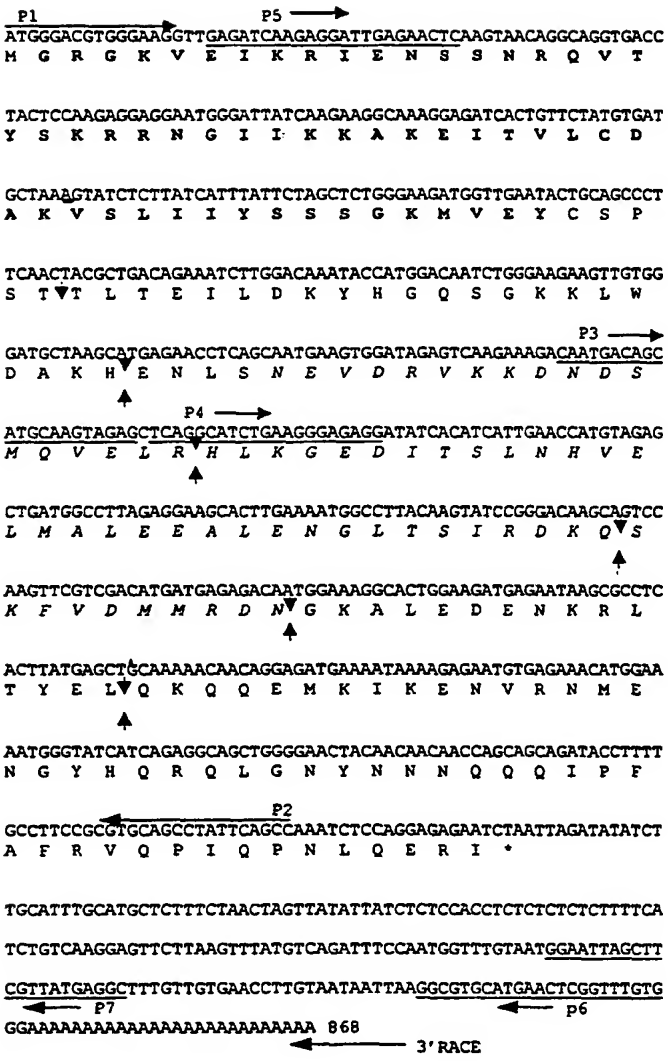


Fig. 1


P1
P5
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TACTCCAAGAGGAGGAATGGGATTATCAAGAAGGCAAAGGAGATCACTGTTCTATGTGAT
Y S K R R N G I I K K A K E I T V L C D
GCTAAAGTATCTCTTATCATTTATTCTAGCTCTGGGAAGATGGTTGAATACTGCAGCCCT
A K V S L I I Y S S S G K M V E Y C S P
TCAACTACGCTGACAGAAATCTTGGACAAATACCATGGACAATCTGGGAAGAAGTTGTGG
S T T L T E I L D K Y H G Q S G K K L W
P3
GATGCTAAGCATGAGAACCTCAGCAATGAAGTGGATAGAGTCAAGAAAGACAATGACAGC
D A K H E N L S N E V D R V K K D N D S
P4
ATGCAAGTAGAGCTCAGGCATCTGAAGGGAGAGGATATCACATCATTGAACCATGTAGAG
M Q V E L R H L K G E D I T S L N H V E
CTGATGGCCTTAGAGGAAGCACTTGAAAATGGCCTTACAAGTATCCGGGACAAGCAGTCC
L M A L E E A L E N G L T S I R D K Q S
AAGTTCGTCGACATGATGAGAGACAATGGAAAGGCACTGGAAGATGAGAATAAGCGCCTC
K F V D M M R D N G K A L E D E N K R L
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T Y E L Q K Q Q E M K I K E N V R N M E
AATGGGTATCATCAGAGGCAGCTGGGGAACTACAACAACAACCAGCAGCAGATACCTTTT
N G Y H Q R Q L G N Y N N N Q Q Q I P F
P2
GCCTTCCGCGTGCAGCCTATTCAGCCAAATCTCCAGGAGAGAATCTAATTAGATATATCT
A F R V Q P I Q P N L Q E R I *
TGCATTTGCATGCTCTTTCTAACTAGTTATATTATCTCTCCACCTCTCTCTCTCTTTTCA
TCTGTCAAGGAGTTCTTAAGTTTATGTCAGATTTCCAATGGTTTGTAATGGAATTAGCTT
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P7
P6
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3'RACE

Fig. 2

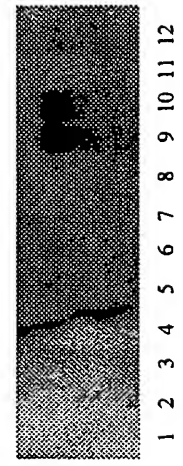
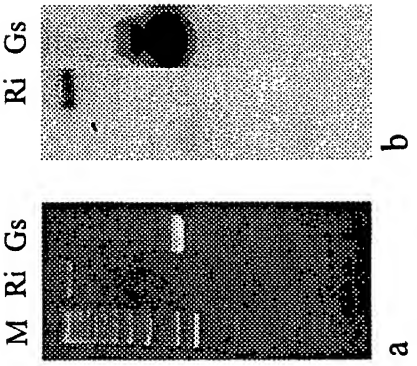


Fig. 3



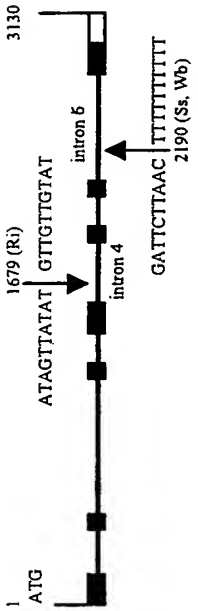


Fig. 5

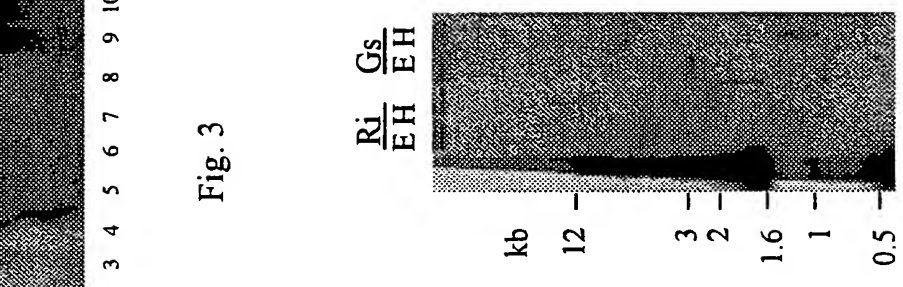


Fig. 4

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Y  S  K  R  R  N  G  I  F  K  K  A  Q  E  L  T  V  L  C  D

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T  T  T  T  K  S  M  Y  D  D  Y  Q  K  T  M  G  I  D  L  W

AGGACACACGAGGAGTCGATGAAAGACACCTTGTGGAAGTTGAAAGAGATCAACAATAAG
R  T  H  E  E  S  M  K  D  T  L  W  K  L  K  E  I  N  N  K

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CTGGCTTCTCTTGACGATGAGATGCAGTCTTCCTTGGATGCCATACGTCAAAGGAAGTAC
L  A  S  L  D  D  E  M  Q  S  S  L  D  A  I  R  Q  R  K  Y

CATGTGATCAAAACTCAGACGGAGACCACCAAGAAGAAGGTTAAGAACTTGGAGCAAAGA
H  V  I  K  T  Q  T  E  T  T  K  K  K  V  K  N  L  E  Q  R

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AACTTGTACACTTTCCACCTCCACCACCCTAACCTCCACCACGGAGGAAGCTCGCTCGGC
N  L  Y  T  F  H  L  H  H  P  N  L  H  H  G  G  S  S  L  G

TCCTCCATTACTCATCTGCACGATCTCCGCCTTGCTTGATCGTGATCTGAGATATGATTA
S  S  I  T  H  L  H  D  L  R  L  A  *

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Fig. 6

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aag gag atc act gtt cta tgt gat gct aaa gta tct ctt atc att tat   144
Lys Glu Ile Thr Val Leu Cys Asp Ala Lys Val Ser Leu Ile Ile Tyr
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tct agc tct ggg aag atg gtt gaa tac tgc agc cct tca act acg ctg   192
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aca gaa atc ttg gac aaa tac cat gga caa tct ggg aag aag ttg tgg   240
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atc aca tca ttg aac cat gta gag ctg atg gcc tta gag gaa gca ctt   384
Ile Thr Ser Leu Asn His Val Glu Leu Met Ala Leu Glu Glu Ala Leu
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cag gag ctc acc gtt ctc tgt gat gcc aag gtc tcc ctc att atg ctc  144
Gln Glu Leu Thr Val Leu Cys Asp Ala Lys Val Ser Leu Ile Met Leu
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tcc aac act aat aaa atg cac gag tat atc agc cct acc act acg acc  192
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aag agt atg tat gat gac tat cag aaa act atg ggg atc gat ctg tgg  240
Lys Ser Met Tyr Asp Asp Tyr Gln Lys Thr Met Gly Ile Asp Leu Trp
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Arg Thr His Glu Glu Ser Met Lys Asp Thr Leu Trp Lys Leu Lys Glu
                85                  90                  95

atc aac aat aag ctg agg aga gag atc agg cag agg ttg ggc cat gat  336
Ile Asn Asn Lys Leu Arg Arg Glu Ile Arg Gln Arg Leu Gly His Asp
            100                 105                 110

cta aat ggc ctg agc ttt gac gag ctg gct tct ctt gac gat gag atg  384
Leu Asn Gly Leu Ser Phe Asp Glu Leu Ala Ser Leu Asp Asp Glu Met
        115                 120                 125

cag tct tcc ttg gat gcc ata cgt caa agg aag tac cat gtg atc aaa  432
Gln Ser Ser Leu Asp Ala Ile Arg Gln Arg Lys Tyr His Val Ile Lys
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act cag acg gag acc acc aag aag aag gtt aag aac ttg gag caa aga  480
Thr Gln Thr Glu Thr Thr Lys Lys Lys Val Lys Asn Leu Glu Gln Arg
145                 150                 155                 160

aga gga aac atg ctg cat ggc tat ttt gac cag gaa gca gcc ggc gag  528
Arg Gly Asn Met Leu His Gly Tyr Phe Asp Gln Glu Ala Ala Gly Glu
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gat cca cag tat ggt tat gag gac aat gag gga gac tac gaa tct gca  576
Asp Pro Gln Tyr Gly Tyr Glu Asp Asn Glu Gly Asp Tyr Glu Ser Ala
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Ser Asn Thr Asn Lys Met His Glu Tyr Ile Ser Pro Thr Thr Thr Thr
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Lys Ser Met Tyr Asp Asp Tyr Gln Lys Thr Met Gly Ile Asp Leu Trp
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Gln Ser Ser Leu Asp Ala Ile Arg Gln Arg Lys Tyr His Val Ile Lys
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Thr Gln Thr Glu Thr Thr Lys Lys Lys Val Lys Asn Leu Glu Gln Arg
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Arg Gly Asn Met Leu His Gly Tyr Phe Asp Gln Glu Ala Ala Gly Glu
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Asp Pro Gln Tyr Gly Tyr Glu Asp Asn Glu Gly Asp Tyr Glu Ser Ala
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180 185 190
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00176

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A01H 5/08; C12N 15/29.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

SEE ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SEE ELECTRONIC DATABASE BOX BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

ChemAbs, Medline, WPIDS: seedless, parthenocarpic, fruit, gene, transgenic, mads, MdPI, MdAP3.
EMBL, Genbank, SwissProt, PIR: Sequence IDs 1-4.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	VAROQUAUX, F, "Less is better: new approaches for seedless fruit production" *Trends in Biotechnology*, vol. 18, p. 223-242, June 2000.	
A	FICCADENTI, N. "Genetic engineering of parthenocarpic fruit development in tomato" *Molecular Breeding*, vol. 5, pp 463-470, 1999.	

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2000	29 DEC 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **PHILIPPA WYRDEMAN** Telephone No : (02) 6283 2554